RESEARCH ARTICLE

Photoacoustic treatment mitigates cognitive dysfunction in a model of sleep-wake rhythm disturbance

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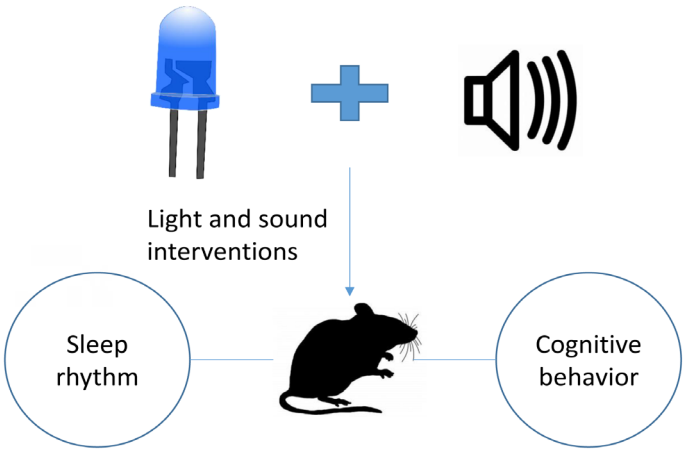
Graphical Abstract

Photoacoustic treatment effectively corrects the abnormal sleep-wake pattern and improves the cognitive dysfunction in sleep-wake rhythm disturbance induced by sleep deprivation



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Abstract

Sleep-wake rhythm disturbances, which are characterized by abnormal sleep timing or duration, are associated with cognitive dysfunction. Photoacoustic treatments including light and sound stimulation have been found to be effective in modulating sleep patterns and improving cognitive behavior in abnormal sleep-wake pattern experiments. In this study, we examined whether light and sound interventions could reduce sleep-wake pattern disturbances and memory deficits in a sleep rhythm disturbance model. We established a model of sleep rhythm disturbance in C57BL/6J mice via a sleep deprivation method involving manual cage tapping, cage jostling, and nest disturbance. We used a Mini Mitter radio transmitter device to monitor motor activity in the mice and fear conditioning tests to assess cognitive function. Our results indicated that an intervention in which the mice were exposed to blue light (40-Hz flickering frequency) for 1 hour during their subjective daytime significantly improved the 24-hour-acrophase shift and reduced the degree of memory deficit induced by sleep deprivation. However, interventions in which the mice were exposed to a 40-Hz blue light at offset time or subjective night time points, as well as 2 Hz-blue light at 3 intervention time points (subjective day time, subjective night time, and offset time points), had no positive effects on circadian rhythm shift or memory deficits. Additionally, a 2000-Hz sound intervention during subjective day time attenuated the 24-hour-acrophase shift and memory decline, while 440-Hz and 4000-Hz sounds had no effect on circadian rhythms. Overall, these results demonstrate that photoacoustic treatment effectively corrected abnormal sleep-wake patterns and cognitive dysfunction associated with sleep-deprivation-induced disturbances in sleep-wake rhythm. All animal experiments were approved by the Experimental Animal Ethics Committee of Drum Tower Hospital Affiliated to the Medical College of Nanjing University, China (approval No. 20171102) on November 20, 2017.

Key Words: *circadian rhythm; cognitive impairment; fear conditioning; light intervention; photoacoustic treatment; rhythm disturbance; rhythm shift; sleep deprivation; sleep-wake rhythm; sound intervention*

Chinese Library Classification No. *R454.2; R363; R364*

Introduction

Millions of people globally are at work during periods in their circadian rhythm when sleep propensity is high, and thus must attempt to sleep when sleep propensity is low (Gronli et al., 2017). Although shift work is a common phenomenon in modern society (Marti et al., 2016), night-shift workers still frequently suffer from sleep rhythm disturbances (Boivin and Boudreau, 2014).

Numerous studies have shown that sleep rhythm disturbances can cause deficits in cognitive function, both in humans and animals (Marshall and Born, 2007; Machi et al., 2012; Han et al., 2019; Li et al., 2019). Disturbed sleep rhythm may also impact disease risk. For instance, acute sleep deprivation can cause an increase in interstitial fluid Aβ in the brain, indicating that the sleep-wake cycle may play a role in the pathogenesis of Alzheimer's disease (Kang et al., 2009). Further, working the night shift might decrease cognitive performance in ICU physicians (Maltese et al., 2016). Long-term sleep rhythm disturbances experienced by travellers who frequently change time zones have been linked with temporal lobe atrophy and spatial cognitive deficits (Cho, 2001). This is supported by the finding that sleep-wake rhythm phase shifts induced by long-term isoflurane anesthesia impaired spatial memory in mice (Xia et al., 2016). Therefore, restoring the sleep-wake rhythm could have powerful potential in solving cognitive problems mediated by sleep rhythm disturbances. Previously, we found that long-term melatonin pretreatment was able to restore the sleep-wake rhythm and improve cognitive behavior (Song et al., 2018). Although melatonin can improve sleep-wake rhythm disturbances, the treatment effects take time to appear and may be accompanied by an adverse effect on mood. Thus, our goal is to find a short-term effective intervention without adverse effects that can restore the normal sleep rhythm and cognitive behavior.

The sleep-wake rhythm is mainly controlled by the master circadian clock, which is located in the hypothalamic suprachiasmatic nucleus (Takahashi et al., 2008). Light is the main cue that synchronizes the circadian clock to the outside world. In humans, light therapy in the morning can advance the sleep-wake cycle in individuals with a delayed sleep phase (Geerdink et al., 2016). In addition to synchronizing the sleep-wake rhythm, bright light has also been found to have a positive effect on cognition and behavior in AD patients (Mitolo et al., 2018). In healthy older adults, exposure to blue-enriched light improved cognitive behavior in the morning (Scheuermaier et al., 2018). In rodents, light pulses with a gamma frequency reduced amyloid deposits (Laccarino et al., 2016), while low-level light therapy enhanced memory retention (Rojas et al., 2012). In addition, sound stimulation has been found to enhance memory consolidation in humans (Leminen et al., 2017) and listening to relaxing music before sleep improved sleep parameters in some patients (Cordi et al., 2019). Therefore, light and sound stimulation may be useful treatments for sleep rhythm disruptions leading to cognitive impairment.

In this study, we established a rhythm disturbance model in mice. By examining gross motor activity patterns and fear conditioning memory after sleep deprivation, we investigated the impact of light and sound interventions on circadian rhythm disruptions and memory impairment.

Materials and Methods

Animals

For the experiments, we used adult specific-pathogen-free C57BL/6J male mice that were 8 weeks old and weighed 20–25 g. Approximately 258 mice were provided by the Model Animal Research Center of Nanjing University, China (license No. SYXK (Su) 2014-0052). Mice were housed under a 12-hour light-dark cycle (lights on at 8:00 and lights off at 20:00) for at least 3 weeks. The mice had free access to water and food throughout the experiments, and were kept at constant room temperature. All animal experiments were approved by the Experimental Animal Ethics Committee of Drum Tower Hospital Affiliated to the Medical College of Nanjing University, China (approval No. 20171102) on November 20, 2017.

We conducted four experiments: model establishment, light intervention, sound intervention, and photoacoustic treatment. Each experiment included gross motor activity circadian rhythm tests and fear conditioning tests. The groups in the four experiments are shown in **Table 1**.

Sleep deprivation

On the 3 days before the sleep deprivation intervention, each animal was gently handled for 1–2 minutes daily using the same interventions used during sleep deprivation: manual cage tapping, cage jostling, and nest disturbance (Tudor et al., 2016). Sleep-deprivation mice were handled for 5 hours from ZT5 onwards in their home cages. Non-sleep-deprived mice were left undisturbed in their home cages (Vecsey et al., 2009). A phase disturbance in the sleep-wake rhythm indicates successful establishment of the sleep deprivation model.

Light and sound interventions

The light or/and sound intervention lasted 1 hour starting at ZT 14 (subjective day), ZT 11.5 (offset time), or ZT 2 (subjective night) after sleep deprivation. The flicker frequency and light intensity of an LED light (blue light bar) was set via RH-LED (version 3.01, Dori, Loudi, Hunan, China) on a computer, which could be connected to a LED controller linked with the LED light bar (Dori, China). The light intensity was set to approximately 78 lux via a light meter and the flicker frequency was set to 40 Hz or 2 Hz. The sound frequency was set to 440 Hz, 2000 Hz, or 4000 Hz via Adobe Audition CC on a computer and the sound intensity was set to approximately 50 dB via a Digital Sound Level Meter AS804 (SMART SENSOR, Shenzhen, Guangdong Province, China). The photoacoustic treatment comprised a combination of light and sound interventions. The stimulation parameters were 40 Hz light combined with 2000 Hz sound performed during subjective day. These parameters were chosen based on the results of light and sound intervention experiments.

Table 1 Group designation in experiments

Group	Intervention and number
Cont	Control (n = 46)
RD	Rhythm disturbance (n = 45)
RD + LT40-D	Rhythm disturbance + 40 Hz-light intervention at subjective day (n = 15)
RD + LT40-N	Rhythm disturbance + 40 Hz-light intervention at subjective night (n = 9)
RD + LT40-O	Rhythm disturbance + 40 Hz-light intervention at offset time (n = 9)
LT40-D	40 Hz-light intervention at subjective day (n = 6)
LT40-N	40 Hz-light intervention at subjective night (n = 6)
LT40-O	40 Hz-light intervention at offset time (n = 6)
RD + LT2-D	Rhythm disturbance + 2 Hz-light intervention at subjective day (n = 9)
RD + LT2-N	Rhythm disturbance + 2 Hz-light intervention at subjective night (n = 9)
RD + LT2-O	Rhythm disturbance + 2 Hz-light intervention at offset time group (n = 9)
LT2-D	2 Hz-light intervention at subjective day (n = 6)
LT2-N	2 Hz-light intervention at subjective night (n = 6)
LT2-O	2 Hz-light intervention at offset time (n = 6)
RD + S440-D	Rhythm disturbance + 440 Hz-sound intervention at subjective day (n = 9)
RD + S2000-D	Rhythm disturbance + 2000 Hz-sound intervention at subjective day (n = 16)
RD + S4000-D	Rhythm disturbance + 4000 Hz-sound intervention at subjective day (n = 9)
S440-D	440 Hz-sound intervention at subjective day (n = 6)
S2000-D	2000 Hz-sound intervention at subjective day (n = 8)
S4000-D	4000 Hz-sound intervention at subjective day (n = 6)
RD + PT-D	Rhythm disturbance + photoacoustic treatment at subjective day (n = 9)
PT-D	Photoacoustic treatment at subjective day (n = 6)

Gross motor activity as a measure of circadian rhythm

Before sleep deprivation, we monitored the circadian activity of the mice for 7 consecutive days. On day 8, the mice were handled from ZT5 to ZT10. We then monitored circadian activity for the next 7 days. We measured the gross motor activity rhythm of the mice via radio transmitter devices (G2 E-Mitter; Mini Mitter Co., Inc., Respironics Company, Bend, Ore, USA). G2 E-Mitters were implanted into the abdominal cavity of mice under sodium pentobarbital anesthesia (50 mg/kg, intraperitoneally). Signals regarding gross motor activity and core body temperature from each mouse were sent to a receiver board (ER-4000 receiver) under the individual cages. The data were recorded via the VitalView Data Acquisition System (version 4.2; Respironics Company) in 6-minute intervals on a computer connected to the receivers. After implantation surgery, the mice were allowed to adapt for at least 14 days before the experiments. Daily gross motor activity for each mouse was fitted to a cosine curve for rhythm analysis. The 24-hour acrophase was analyzed using the cosine curve for each day, which showed the peak point of activity. Acrophase changes indicated circadian rhythm shifts.

Fear conditioning tests

In the fear conditioning experiments, during the training period, each mouse was placed in a chamber. After a 3-minute adaptation for environment, we presented a 30-second sound tone (90 dB, 2000 Hz) accompanied by a 0.8 mA foot shock during the final 2 seconds. Each mouse stayed in the same chamber for 1 minute before being returned to the home cage. After 24 hours, animals underwent contextual memory and cue memory tests. During the contextual memory test, each mouse was placed in the same chamber as on the previous day for 3 minutes. The percentage of freezing time (Freezing %) during the 3 minutes was used as a measure of contextual memory. In the cue memory test, we altered the chamber environment by changing the chamber wall and ground. After a 3-minute adaptation period in the new environment, we presented a 30-second sound tone identical to that heard on the previous day, but did not deliver a foot shock. Afterwards, the mice stayed in the chamber for 1 minute before being returned to the home cage. The percentage of freezing time (Freezing %) during the 30 seconds in the chamber was used as a measure of cue memory. Freezing % was equal to (freezing time/total time) × 100%. All procedures were coordinated via PACKWIN (Panlab, Harvard Apparatus, USA) on a computer connected to the device, and data were analyzed using the same software.

Statistical analysis

Statistical analysis was carried out using SPSS 22.0 software (IBM Corporation, Armonk, NY, USA). All data are expressed as the mean ± SD. The data from fear conditioning experiments were analyzed using a one-way analysis of variance followed by Bonferroni *post hoc* test to detect the differences among the experimental groups. Unpaired two-tailed Student's *t*-tests were used to examine differences between the two groups.

The Mini Mitter data were examined with respect to the 24-hour acrophase via a repeated measures analysis of variance. We conducted *post hoc* analyses using a Student's *t*-test with the Bonferroni correction to compare the various test data with those collected on the day before sleep deprivation in each group. Differences were considered statistically significant when the P value was less than 0.05.

Results

Influence of sleep deprivation in mice

First, we determined that 5 hours of sleep deprivation led to an animal model of rhythm disturbance (RD), as shown in **Figure 1A**. The circadian rhythm parameters, as reflected by the 24-hour acrophase, had changed within 1 day after sleep deprivation. Specifically, the 24-hour acrophase was significantly delayed on day 1 compared with the day before sleep deprivation (2.68 ± 0.13 hours *vs.* –0.30 ± 0.16 hours, P = 0.001) in the RD group. Therefore, 5 hours of sleep deprivation can cause a circadian rhythm phase-shift.

In the fear conditioning contextual test, we found that the percentage of freezing time in the novel environment was significantly lower in the RD group compared with the

control group (39.64 ± 7.75% *vs.* 72.96 ± 8.68%, $P < 0.01$). In the cue test, the RD mice also showed a lower percentage of freezing time (46.47 ± 9.07% *vs.* 61.78 ± 10.42%, $P = 0.038$; **Figure 1B**). These results indicate that fear conditioning memory was impaired in the RD group. Thus, sleep deprivation via 5 hours of handling appears to have impaired both the circadian rhythm and memory in mice.

Effect of different light interventions on RD mice

We performed our intervention at three time points: subjective day, subjective night, and offset time. Stimuli were 40-Hz and 2-Hz blue light. The effects of the light stimuli on circadian rhythms are shown in **Figure 2A** and **B**. The effects of the light stimuli on fear conditioning memory are shown in **Figure 2C** and **D**.

Effect of light interventions on sleep-wake rhythm

As shown in **Figure 2A**, we found that exposure to a 40-Hz blue light during subjective day significantly attenuated sleep rhythm disturbances on day 1, while exposure to the other two stimuli did not modulate the size of phase-shifts compared with the day before sleep deprivation ($P = 0.037$ on day 1 in the RD + LT40-N group; $P = 0.047$ on day 1, $P = 0.023$ on day 2 in the RD + LT40-O group). As shown in **Figure 2B**, the 2-Hz blue light intervention did not ameliorate the rhythm disturbance.

Effect of light interventions on fear conditioning memory

As shown in **Figure 2C**, in the contextual memory tests, the percentage of freezing time was significantly lower in the RD group compared with the control group (22.37 ± 10.79% *vs.* 71.97 ± 10.04%, $P < 0.05$), which was consistent with our findings regarding the influence of sleep deprivation. Freezing behavior was improved in the RD + LT40-D group compared with the RD group (69.57 ± 9.86% *vs.* 22.37 ± 10.79%, $P < 0.05$) and the non-RD groups. We also found that interventions during the subjective day improved cue memory in RD mice (58.44 ± 5.67% *vs.* 32.56 ± 6.38%, $P < 0.05$). Conversely, interventions at the other two time points elicited no improvement in contextual or cue memory in RD mice. For the 2-Hz blue light intervention, we found no evidence of improvements in fear conditioning in any of the groups compared with the RD group (**Figure 2D**).

These results indicate that the 40-Hz blue light intervention during subjective day improved the phase shift and memory impairment induced by sleep deprivation. Thus, subjective day appears to be an effective time point for interventions.

Effect of different sound interventions on RD mice

Because the results of the light interventions suggested that subjective day was an appropriate time point to perform interventions, we also performed sound interventions at this time point. The effects of the sound intervention on circadian rhythms are presented in **Figure 3A**, and the effects of the sound intervention on fear conditioning memory are shown in **Figure 3B–D**.

Effect of different sound interventions on sleep-wake rhythm

The 2000-Hz sound intervention ameliorated the phase shift in RD mice on day 1, while this was not the case for the 440-Hz and 4000-Hz sound interventions (**Figure 3A**). Mice in the 440-Hz and 4000-Hz groups still showed a phase-shift on day 1. Therefore, the 2000-Hz sound intervention appears to have attenuated the rhythm disturbance in sleep deprived mice.

Effect of sound intervention on fear conditioning memory

As shown in **Figure 3B**, the 440-Hz sound intervention did not improve the impaired memory in RD mice. In the fear conditioning tests, the 2000-Hz sound intervention significantly improved cue memory. Freezing behavior was longer in the RD + S2000-D group compared with the RD group in the cue test (89.62 ± 6.77% *vs.* 70.56 ± 10.56%, $P = 0.001$). In the contextual test, no statistically significant changes were detected between the RD + S2000-D and RD groups (**Figure 3C**). The 4000-Hz sound intervention also failed to improve memory. As shown in **Figure 3D**, freezing behavior in both tests was lower in the RD + S4000-D group compared with the control group and we found no statistically significant changes between the RD + S4000-D and RD groups.

The 2000-Hz sound intervention improved the circadian rhythm phase shift and partial memory deficit induced by sleep deprivation. Therefore, 2000-Hz sound treatment should be considered for sleep deprivation-related memory and circadian rhythm disturbances.

Effect of photoacoustic treatment on RD mice

Because the 40-Hz blue light and 2000-Hz sound interventions both improved rhythm shift and memory deficits, we sought to determine whether a light-sound compound intervention would have similar or even greater positive effect. We found that photoacoustic treatment also attenuated the circadian rhythm disturbance (**Figure 4A**) such that the 24-hour acrophase was not significantly different on day 1 compared with the day before sleep deprivation.

As exhibited in **Figure 4B** and **C**, light and sound interventions improved freezing behavior in the contextual test and cue test, which was consistent with the data shown in **Figures 2** and **3**. Memory was improved in the RD + PT-D group in the contextual test (59.28 ± 12.25% *vs.* 30.61 ± 9.66%, $P = 0.016$) and cue test (85.00 ± 6.07% *vs.* 63.35 ± 9.69%, $P < 0.01$). Although photoacoustic treatment alleviated the memory deficit induced by sleep deprivation, this effect was not greater than that of the light or sound interventions delivered separately.

Taken together, our data indicate that the photoacoustic treatment, as well as separate light or sound stimulation, improved the phase shift and memory impairment induced by sleep deprivation (**Figure 5**).

Discussion

In this study, we examined disruptions in circadian rhythms and impaired fear conditioning memory induced by 5 hours

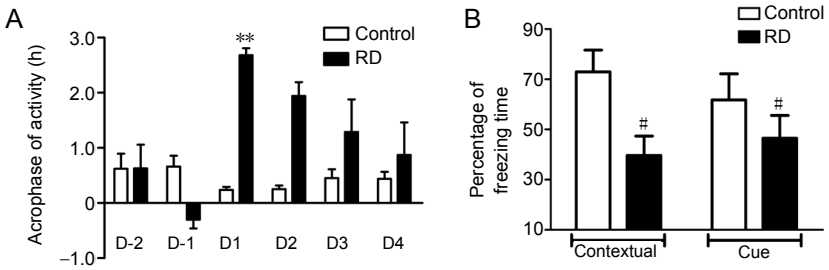

Figure 1 Circadian rhythm phase shift on day 1 and memory impairment induced by 5-hour-sleep deprivation.
(A) Sleep deprivation-induced changes in circadian rhythms. The figure shows the 24-hour-acrophase on day 1 compared with that on the day before sleep deprivation (Day-1, D-1). Data are presented as the mean ± SD. $^{**}P < 0.01$, *vs.* Day-1 (D-1) in the RD group (mean ± SD, $n = 3$; repeated measures analysis of variance). (B) Five hours of sleep deprivation induced a fear conditioning memory impairment on day 1. $^{\#}P < 0.05$, *vs.* control group (mean ± SD, $n = 5$, Student's *t*-test). RD: Rhythm disturbance.

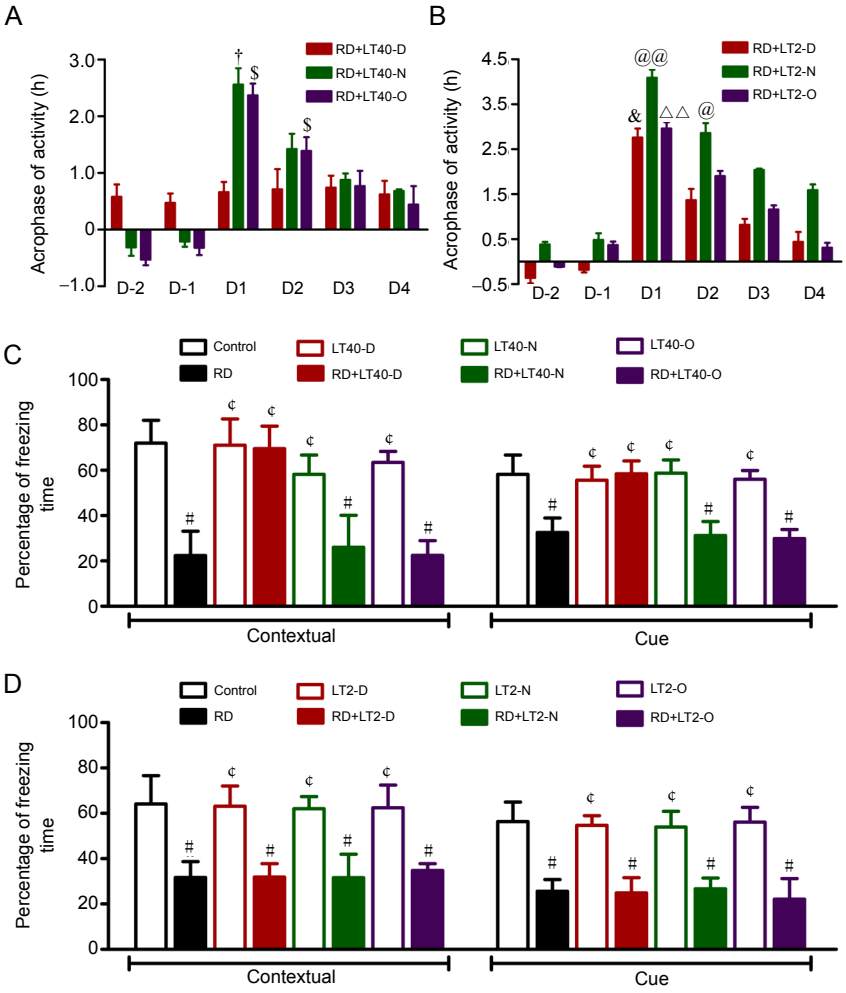

Figure 2 Effect of blue light intervention on circadian rhythm and memory.
(A) The effect of a 40-Hz blue flickering light intervention at 3 time points on the 24-hour acrophase in rhythm-disturbed mice. $^{\dagger}P < 0.05$, *vs.* the day before sleep deprivation (Day-1, D-1) in the rhythm-disturbed + 40-Hz light intervention at subjective night group (RD + LT40-N); $^{\$}P < 0.05$, *vs.* Day-1 (D-1) in the rhythm-disturbed + 40-Hz light intervention at offset time group (RD + LT40-O). (B) The effect of a 2-Hz blue flickering light intervention at 3 time points on the 24-hour acrophase of rhythm-disturbed mice. $^{\&}P < 0.05$, *vs.* Day-1 (D-1) in the rhythm-disturbed + 2-Hz light intervention at subjective day group (RD + LT2-D); $^{@}P < 0.05$, $^{@@}P < 0.01$, *vs.* Day-1 (D-1) in the rhythm-disturbed + 2-Hz light intervention at subjective night group (RD + LT2-N); $^{\Delta\Delta}P < 0.01$, *vs.* Day-1 (D-1) in the rhythm-disturbed + 2-Hz light intervention at offset time group (RD + LT2-O). (C, D) Different effects of 40-Hz blue light and 2-Hz blue light on behavior in the fear-conditioning test at three time points, respectively. $^{\#}P < 0.05$, *vs.* control group; $^{¢}P < 0.05$, *vs.* rhythm disturbance group (RD). Data are presented as the mean ± SD (A, B: $n = 3$, repeated measures analysis of variance; C, D: $n = 6$, one-way analysis of variance followed by Bonferroni *post hoc* test). RD: Rhythm disturbance.

of sleep deprivation. We measured the effects of different frequencies of light and sound interventions at three time points in a rhythm disturbance model. We found that a short period of sleep deprivation caused a slight phase shift in the circadian rhythm and deficits in fear conditioning memory, which could be improved by a short pulse of blue light flickering at 40 Hz, a 2000 Hz sound, and photoacoustic treatment during the subjective day on the day (ZT14) after sleep deprivation.

Our model of sleep rhythm disturbance, induced by sleep deprivation, led to a shift in sleep-wake rhythm, contextual memory impairment, and cue memory impairment, consistent with previous studies (Hagewoud et al., 2010; Kumar and Jha, 2012). In recent years, low-level light therapy has become a popular intervention for several diseases, such as seasonal affective disorder, sleep/wake disturbances, and cognitive dysfunction (Friedman et al., 2012; Rojas et al., 2012; Meesters et al., 2016). Light can regulate circadian

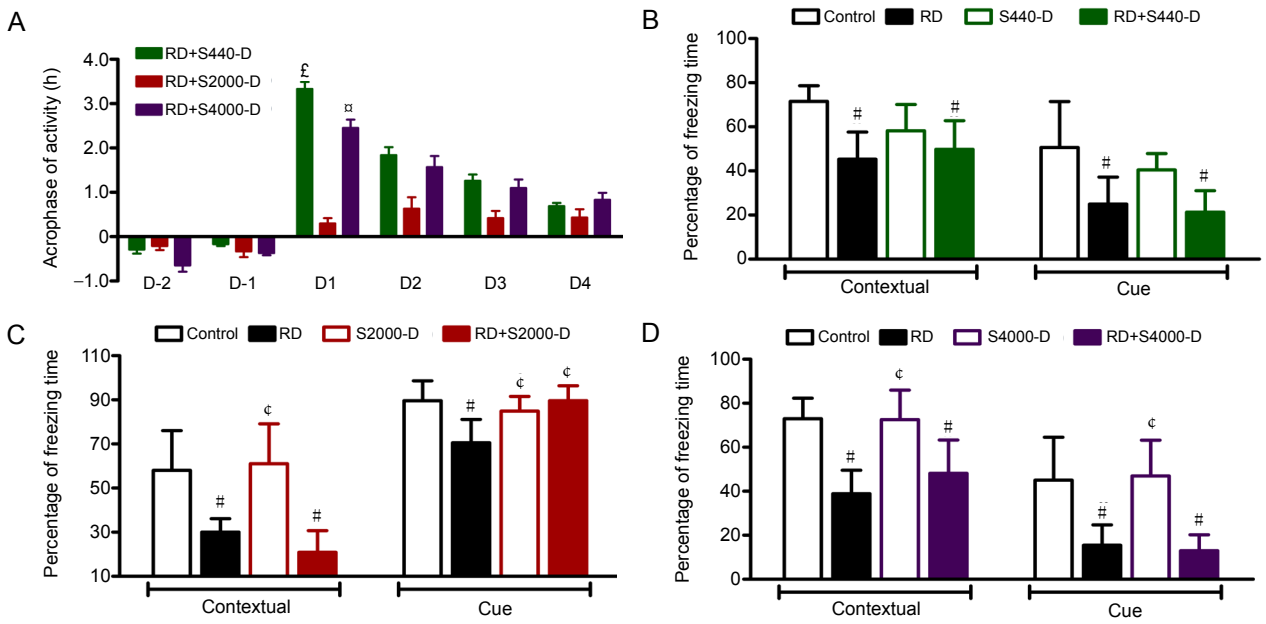

Figure 3 Effect of sound intervention on circadian rhythm and memory.
(A) Effect of 440 Hz, 2000 Hz, and 4000 Hz sound interventions on the 24-hour acrophase in rhythm-disturbed mice. £$P < 0.05$, *vs.* the day before sleep deprivation (Day-1, D-1) in the rhythm-disturbed + 440-Hz sound intervention group (RD + S440-D); ¤$P < 0.05$, *vs.* Day-1 (D-1) in the rhythm-disturbed + 4000-Hz sound intervention group (RD + S4000-D) (mean ± SD, $n = 3$; repeated measures analysis of variance). (B) Effect of the 440-Hz sound intervention on performance in the fear conditioning test. #$P < 0.05$, *vs.* control group (mean ± SD, $n = 6$, one-way analysis of variance followed by *Bonferroni's post-hoc* test). (C) Effect of 2000-Hz sound intervention on performance in the fear-conditioning test. $n_{Cont} = 8$, $n_{RD} = 7$, $n_{S2000\text{-}D} = 8$, $n_{RD+S2000\text{-}D} = 7$. #$P < 0.05$, *vs.* control group; ¢$P < 0.05$, *vs.* rhythm disturbance group (RD) (mean ± SD; one-way analysis of variance followed by Bonferroni *post hoc* test). (D) Effect of 4000-Hz sound intervention on performance in the fear conditioning test. #$P < 0.05$, *vs.* control group, ¢$P < 0.05$, *vs.* rhythm disturbance group (RD) (mean ± SD, $n = 6$; one-way analysis of variance followed by *Bonferroni's post-hoc* test). RD: Rhythm disturbance.

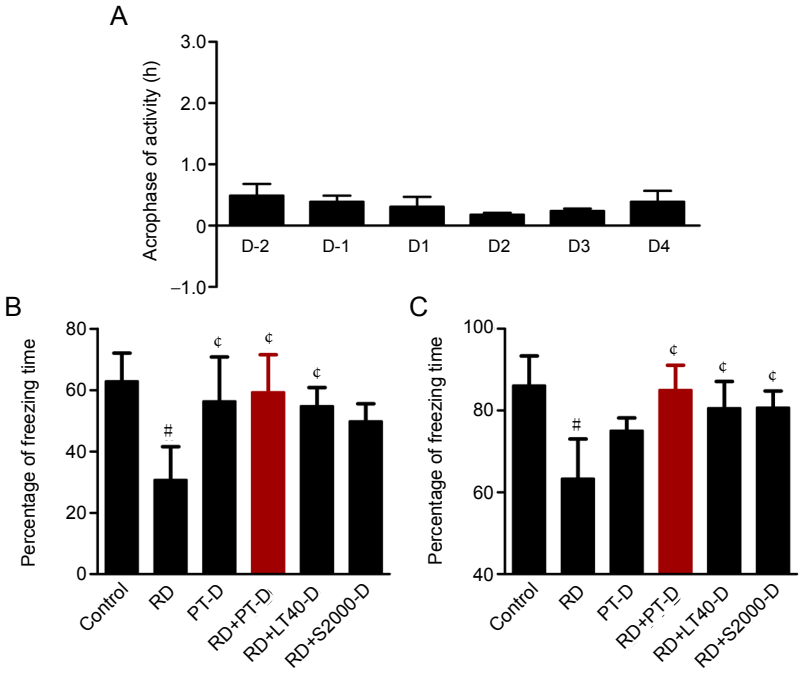

Figure 4 Effect of photoacoustic treatment on circadian rhythm and memory.
(A) Effect of photoacoustic treatment on the 24-hour acrophase in rhythm-disturbed mice. Data are presented as the mean ± SD ($n = 3$; repeated measures analysis of variance). D-2 refers to two days before sleep deprivation, D-1 is the day before sleep deprivation, and D1–4 refers to the first, second, third, and fourth days after sleep deprivation. (B, C) Different effects of 40-Hz light intervention, 2000-Hz sound intervention, and photoacoustic treatment on contextual and cue memory, respectively. #$P < 0.05$, *vs.* control group; ¢$P < 0.05$, *vs.* rhythm disturbance group (RD) (mean ± SD, $n = 6$, one-way analysis of variance followed by Bonferroni *post hoc* test). RD: Rhythm disturbance.

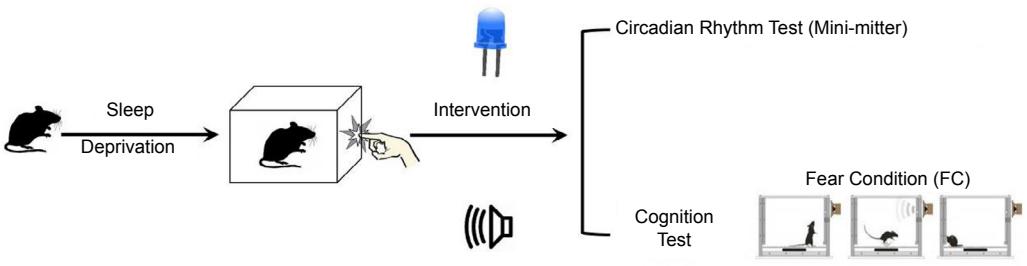

Figure 5 Diagram of the experimental design.
After sleep deprivation, mice received a light or sound intervention before a circadian rhythm test or cognition test. Results showed that blue light and sound interventions had a positive effect on rhythm-disturbed mice.

rhythms and sleep in mammals (Freedman et al., 1999; Lupi et al., 2008). Further, a pilot study suggested that short-term exposure to bright light might have beneficial effects on cognitive function in humans (Bersani et al., 2008). Blue light was found to cause arousal and delay sleep onset, and the sleep-promoting effect of blue light might be mediated by the master clock in the suprachiasmatic nucleus (Pilorz et al., 2016). Additionally, green light might induce rapid sleep onset, opposite to the effect of blue light (Pilorz et al., 2016). In the present study, we wanted examine the effects of light interventions in our rhythm disturbance model. We found that blue light stimulation successfully improved memory and rhythm in RD mice. However, green light did not elicit significant improvements in sleep rhythm disturbance or memory deficits (data not shown).

We found that a 40-Hz flickering blue light (gamma frequency) presented during subjective day after sleep deprivation improved cognitive function and rhythm phase-shift, while this was not the case for 2-Hz light. Laccarino et al. suggested that light pulsing at the gamma frequency attenuates the amyloid load, which is consistent with our results (Laccarino et al., 2016). However, a 40-Hz blue light presented at subjective night or offset time had no effect on behavior or sleep-wake rhythm. In another study, light exposure at inappropriate times had adverse effect (Van der Maren et al., 2018). Therefore, the effects of blue light may be time specific. Subjective day is the time at which sleep propensity is low in mice. After sleep deprivation during the sleep phase in mice, interventions performed during subjective day could prevent the activity phase from being delayed, as well as restrict memory deficits, on the next day.

Various sounds have been found to cause sleep disturbances and other health problems (Tiesler et al., 2013; Onakpoya et al., 2015). However, in our study, regular rhythmic sounds presented at a specific time had a positive effect on sleep disturbances. Low frequency (< 50 Hz) sounds can manipulate sleep spindles, which are associated with many cognitive domains, including memory (Antony and Paller, 2017). Extremely low frequency (around 1 Hz) sounds can enhance slow sleep waves and improve word pair recall in adults (Ngo et al., 2013). Gamma frequency (40 Hz) sound stimulation can improve hippocampus-dependent memory in a mouse AD model (Martorell et al., 2019). However, the effect of relatively high frequency (around 2000 Hz) sound on sleep and memory is still unknown. In our study, 440 Hz, 2000 Hz, and 4000 Hz sounds were presented after sleep deprivation, and only 2000-Hz sounds improved phase shifts and cue memory. In our fear conditioning experiment, a 2000 Hz-sound (30 seconds) appears to have been the interference factor for the sound intervention results. Our data demonstrated that a 2000-Hz sound intervention during subjective day markedly improved rhythm disturbances and cognitive deficits. Thus, this stimulus might be useful in sound therapy applications.

During the light and sound intervention tests, we identified appropriate light and sound conditions, as well as intervention time points. We found that the photoacoustic treatment had a positive effect on sleep-wake rhythms and cognition. However, this effect was not greater than either the 40-Hz light stimulation or 2000-Hz acoustic stimulation. It has been reported that photoacoustic stimulation with an undefined frequency could enhance relaxation and sleep in patients (Tang et al., 2016). We speculate that our photoacoustic stimulation parameters might be useful in the treatment of sleep-wake rhythm disturbance. However, defining the mechanisms underlying the effect of the 40-Hz light and 2000-Hz sound entails further research.

Taken together, our data indicate that 5 hours of sleep deprivation can cause a sleep-wake rhythm phase shift and memory deficits. After sleep deprivation, appropriate light and sound interventions could reverse the cognitive dysfunction and circadian rhythm phase shift. Our findings provide new information relevant to the development of a noninvasive approach to treating sleep-deprivation related cognitive decline and circadian phase shifts.

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